Kidney International, Vol. 46 (1994), pp. 847–854

Calcium oxalate nephrolithiasis, a free or fixed particle disease

DIRK J. KOK and SAEED R. KHAN

Department of Pathology, University of Florida, Gainesville, Florida, USA, and Department of Endocrinology, Academic Hospital Leiden, The Netherlands

Calcium oxalate nephrolithiasis, a free or fixed particle disease. The chances of stone formation occurring through a free particle mechanism were calculated using the approach of Finlayson and Reid [1]. For these calculations we used new data on nephron dimensions, supersaturation and crystal growth rates in urine, and also incorporated the size increasing effect of crystal agglomeration. The calculations were performed assuming different levels of oxalate excretion, simulating the diurnal variation and acute hyperoxaluria following a dietary load. In addition urinary flow conditions were varied according to changes in daily urinary volume. It is shown that during the normal transit time of urine through the nephron, particles can obtain a size big enough to be retained in the nephron. This is mainly due to the size-increasing effect of the agglomeration process. The precipitable amount of oxalate present is not limiting for the maximum attainable particle size. However, acute increases in oxalate excretion do pose a risk because supersaturation is reached earlier in the nephron and consequently the crystal particles are allowed more time to increase in size. In conclusion, the present calculations demonstrate that during the normal transit time through the kidney, crystalline particles can be formed which are large enough to be retained because of their size and thus form the nidus of a stone. The highest risk is encountered at the end of those collecting ducts where crystals formed in nephrons with a long loop of Henle meet and agglomerate.

Supersaturation of the urine with respect to calcium oxalate is a prerequisite for calcium oxalate urinary stone formation. Urinary stones, however, do not form only because the urine is supersaturated. The occurrence of supersaturated urine and crystalluria is a common phenomenon for both stone-forming patients and healthy subjects [2–4]. This finding led to the notion that the precipitation of calcium oxalate monohydrate (COM) by itself poses no problem as long as the particles formed are allowed to pass freely through the urinary tract. Problems occur only when the particles are retained and allowed to form the nidus of a stone. Retention is defined here as movement through the urinary tract at a speed lower than that of the urinary flow. Theoretically retention can occur because the particles become too big to pass freely through the renal tubules, by adhesion to the tubular cells or by trapping of particles at sites of disturbed urinary flow. When the size of a particle is the decisive factor, the process is defined as the free particle mechanism. This is in contrast to the fixed particle mechanism, where adhesion plays a dominant role. Finlayson and Reid [1] calculated the chances of forming a stone through a free particle mechanism in the renal tubules, pelvis and the bladder. The calculations were based on average normal values for oxalate excretion, kidney structure and nephron dimensions. They found that even under the worst possible assumptions, when all oxalate is allowed to precipitate and crystal growth occurs at maximum speed, the crystalline particles could never become large enough to be retained by sheer size in the nephron or the pelvis during normal transit times through the kidney. Only bladder stone formation might be explained by this mechanism. This led to the conclusion that particle retention caused by adherence to cells or disturbed urinary flow conditions must play an important role in stone formation. The validity of these calculations still stands; however, several refinements can be made. New data have become available on the dimensions of the renal tubules through which the particles must pass. There is a significant variation in the thickness of the epithelial cells lining the renal tubules along the length of the nephron [5]. Consequently, the inner diameter of the nephron varies considerably from one section of the nephron to the next. In addition, the length of the nephrons may vary greatly, (compare short loops vs. long loops.) Also, urinary flow is not constant throughout the nephron, but varies for each segment, depending on the size and number of nephrons and the amount of water resorbed up to that point. Precipitation of all oxalate from the urine is unlikely to occur. Precipitation will continue only until equilibrium concentrations have been reached. Crystal growth in urine will not proceed at maximum rate but at a reduced rate. Urinary oxalate excretion shows a wide diurnal variation [6,7]. Especially after ingestion of oxalate rich foods it may be sharply increased [8]. Instead of using only an average daily excretion of oxalate it may thus be more relevant to base the calculations on the extremes encountered during the day. Finally, particle size will be greatly influenced by the occurrence of crystal agglomeration, a process which was not considered in the original calculations. A contributory role for crystal agglomeration in retention is apparent in *in vivo* studies of calcium oxalate nephrolithiasis. When nephrolithiasis was induced by induction of acute hyperoxaluria, the type of particles retained in the renal tubules changed with time [9]. At early time periods both single crystals as well as small agglomerates were seen in the renal tubules. However, only large agglomerates of calcium oxalate monohydrate crystals were still present three days after the hyperoxaluric challenge indicating the importance of agglomeration in crystal retention. We have now repeated these calculations using the more accurate up-to-date data and also incorporated the size increasing effects of crystal agglomeration.

Received for publication November 22, 1993
and in revised form March 25, 1994
Accepted for publication March 28, 1994

Methods

Data used

The data used in the original and present calculations are summarized in Table 1. Validation of (the choice of) data is given below.

Supersaturation and growth rate. Since oxalate is the limiting factor in the precipitation of calcium oxalate from urine, the calculations are based on the pool of oxalate available. In the original calculations all oxalate was allowed to precipitate and the growth rate was taken to be maximal. Some oxalate, however, will remain in solution. In an average normal urine containing 0.44 mmoles oxalate, 4 mmoles calcium and having a relative supersaturation of 5, approximately 0.1 mmoles of oxalate will remain in solution at equilibrium, thus 0.34 mmoles or 77.5% will precipitate out maximally. A further refinement is to correct for the diurnal variation in oxalate excretion. With normal daily excretion rates of oxalate, the highest concentration during the day is twice or more the mean daily concentration [6,7], and after ingestion of oxalate rich food the concentration can go up 289% [8]. We therefore based the calculations on three different scenarios of daily oxalate excretion, low normal (0.031 mM), high normal (0.62 mM) and acute high (0.89 mM) oxalate, with constant calcium excretion. The solubility of calcium oxalate monohydrate in the urine of stone-formers, that is, the amount present at equilibrium, is not different from that in the urine of healthy subjects [10]. Thus, in the three scenarios, the precipitable amount of oxalate is respectively 0.24 mM, 0.54 mM and 0.82 mM. We did not take into consideration that stone formers tend to excrete more calcium, this would increase the precipitable amount of oxalate. As will be shown further, this parameter is not the limiting factor in the determination of the particle size. The maximal COM linear crystal growth rate, as used in the original calculations, is 2×10^{-4} cm per minute. In urine COM. crystal growth is reduced by more than 50% [10]. For the calculations we used a 50% inhibited growth rate, 1.10^{-4} cm per minute.

Transit times. We know the amount of fluid entering the kidneys, 125 ml of serum filtered per minute giving 116 ml of filtrate or 58 ml/min per kidney. In the proximal tubule 65% of the water is reabsorbed. At the end of the proximal tubule thus 35% remains, on average throughout the proximal tubule 67.5% remains. For the descending loop of Henle these numbers are 25%, 10% and 17.5%. Under high diuresis, daily urine volume 16.7 liters, no further resorption is assumed to occur. When the urine is concentrated further, to 1.44 or to 0.6 liters, this occurs in the inner and outer medullary collecting ducts, the degree depending upon the final urinary output. Thus the flow in ml per minute can be calculated for each segment of the nephron (Table 1). Since the internal diameter and the number of the segments are known [11], the flow in mm per second can be calculated as flow (ml per min)/area segment · number of segments. For the proximal tubule, for instance, flow equals 39.1 ml/min, area is 7.1, 10^{-4} mm^2 and number is 1.2 million. The flow in mm/second thus is 0.76. The total length of the proximal tubule is on average 18 mm, thus the transit time is 24 seconds. These numbers compare well to the actual transit time measured in stop-flow studies in dogs under maximum diuresis of 28 seconds [12] and the flow velocity measured in rats using video-fluorescence, 0.48 mm/sec [13]. In Table 1 the transit times of urine through the different segments is given for different situations. These are differences in the length of the descending loop of Henle (short 0 mm, medium 7 mm and long 14 mm), and ascending loop of Henle (short 6 mm, medium 12 mm, long 18 mm) and differences in daily urinary volume (16.7 liters, 1.44 liters and 0.6 liters, respectively). The high volume mimics the situation immediately following ingestion of a large volume of water; 1.44 liters represents the average urinary volume and 0.6 liters the minimum. Under a normal oxalate loading condition the nucleation threshold is surpassed somewhere in the descending loop of Henle due to water resorption [14]. We therefore base our calculations under normal conditions on nucleation starting at the end of the descending loop of Henle, allowing for nucleation lag time in the descending loop of Henle. The passage time for the crystals thus will be the time needed to pass the ascending loop of Henle, distal tubule, outer medullary collecting duct (OMCD), inner medullary collecting duct (IMCD) and duct of Bellini (DOB) (Table 1).

Under acute hyperoxaluria, crystals can already be found in the proximal tubule (Fig. 1) [9, 15, 16]. For this condition, therefore, nucleation can be assumed to start in the proximal tubule. Crystals then have a passage time equal to that for the whole kidney.

Crystal numbers. In the original calculations, the number of particles which can be expected per transit time per collecting duct during crystalluria was taken to be 33.75. This number, which now appears to be too low, was derived as follows. From the data of [2] during crystalluria one can expect approximately 7200 crystals per ml of urine. Assuming a flow rate of 1440 ml per day, or 1 ml per minute, this means 7200 crystals per minute. On average there are 320 ducts of Bellini per kidney and the transit time of particles through the kidney was taken to be three minutes. Thus you may expect 7200 × 3/640—33.75 crystals per duct per transit time. The number 7200 was extracted from the particle size distribution measurements during crystalluria performed by Robertson, and reflected the number of particles in the peak of their measured distribution rate. The actual crystal density, however, will be the sum of the crystal numbers of all particle sizes counted. In [2] at least 50% of the crystals were not detected because they were outside the detection limits. Taking this all in consideration, the actual number of crystals one can expect during crystalluria is 24084 crystals per ml, which translates to 73 to 154 crystals per duct per transit time, depending on the transit time. Moreover crystals are not uniformly distributed throughout the renal tubules. Even under severe hyperoxaluric conditions, the crystals are concentrated in only a few of the renal tubules (Fig. 2). To mimic this effect the calculations were also performed assuming crystals to be present in 50% of the ducts only. Overall three crystalluria scenarios are used, 33.75, 73 to 154 and 146 to 308 crystals per collecting duct.

Tubular dimensions. Originally [1] the restrictive diameter was taken to be 200 μm, which is the outer diameter of the duct of Bellini at the papillary tip. But the actual dimensions that particles encounter are much smaller. First, the opening at the papillary tip is not round but slit-like, as is clearly seen in Figure 3. In this picture the long axis varies from 60 to 120 μm and the small axis from 7 to 23 μm. Second, the inner diameter of the renal tubules which determines the sticking size of a particle is much smaller than the outer diameter. Due to variations in the epithelial thickness, 0.1 to 120 μm, the inner diameter varies greatly along the nephron. The data for human kidneys [11] are not complete enough for our purposes, and therefore we used data for rabbit kidneys [5] which compare reasonably well to human kidneys

Table 1. Data used for calculations

Segment	P	DlH	AlH	D	OMCD	IMCD	DOB
Tubular dimensions							
Outer D, H μ	50–65	14–22	14–22	20–50	50–200	50–200	200
Outer D, R μ	45–65	15–40	20–30	20–60	40–55	55–300	300
Epithelial thickness, R μ	10–15	0.5–1.5	0.3–1.0	0.7–18	5–10	10–120	100–120
Inner D, R μ	25–35	14–37	19–29	18–30	30–35	35–60	60–100
Area $10^{-4}mm^2$	7.1	5.1	4.5	4.5	8.29	17.7	50.2
Length, H *mm*	12–24	0–14	6–18	2–9	16	6	5
Number per kidney	1,200,000	1,200,000	1,200,000	1,200,000	200,000	5,120	320

Segment	P[c]	DlH[a]	AlH[b]	D	OMCD[d]	IMCD[d]	DOB
Flow characteristics							
Resorp.[b] %	65	25	0	0	0/6.8/7.2	0/2.3/2.5	0/0/0
Remain. %	67.5	22.5	10	10	10/6.6/6.4	10/2.0/1.6	10/0.9/0.3
Flow *ml/min*	39.1	13.1	5.8	5.8	5.8/3.8/3.7	5.8/1.0/0.9	5.8/0.5/0.2
Flow n/d 10^{-4} *µl/s*	5.4	1.8	0.8	0.8	4.8/3.2/3.1	189/32/29	3021/259/103
Flow n/d *mm/s*	0.76	0.35	0.18	0.18	0.6/0.4/0.4	10.7/1.8/1.6	60/5.2/2
T, s. LOH *sec*	24	0	33	31	28/41/42	1/3/4	0.1/1/2.5
T, m. LOH *sec*	24	20	67	31	28/41/42	1/3/4	0.1/1/2.5
T, l. LOH *sec*	24	40	100	31	28/41/42	1/3/4	0.1/1/2.5

Load	Total T[a]	F&R	K&K normal	peak	acute
Oxalate concentration *mM*		0.31	0.31	0.62	0.89
Precipitable oxalate *mM*		0.31	0.24	0.54	0.82
Crystal growth rate $cm \cdot min^{-1}$		2×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}
Crystals per minute		7200	7200,24084	7200,24084	7200,24084
Crystals/duct/T[a,e]					
s. loops	117/133/137		73/83/86	73/83/86	73/83/86
m. loops	171/187/191	11.25	107/117/120	107/117/120	107/117/120
l. loops	224/241/245		141/152/154	141/152/154	141/152/154

Data on human kidneys (H) are derived from [11], those on rabbit kidneys (R) from [5]. Abbreviations are: P, proximal; DLH, descending loop of Henle; ALH, ascending loop of Henle; D, distal; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct; DOB, duct of Bellini; F&R, [1]; K&K, [this article].

Transit times are calculated for short (s), medium (m) and long (l) loops of Henle.

[a] Three different situations are given, based on a daily urinary volume of 16.7, 1.44 or 0.6 liters, respectively.

[b] Water resorption over total length of segment, for the calculation of the average % water remaining it is assumed that resorption is uniform over total length.

[c] Plasma filtered is 125 ml/min, filtrate is 116 ml/min or 58 mml/min per kidney.

[d] With maximal flow there is no resorption after the loop of Henle, under other situations additional resorption occurs in OMCD and IMCD.

[e] The calculations are also performed with double these numbers, simulating the situation that only 50% of the collecting ducts contain crystals.

(Table 1). In the rabbit the inner diameter approximates 25 to 35 μm in the proximal tubule, 14 to 37 μm in the loop of Henle, 19 to 29 μm in the distal tubule, 30 to 35 μm in the outer medullary collecting duct, 35 to 60 μm in the inner medullary collecting duct, and 60 to 100 μm for the duct of Bellini.

Agglomeration. Crystal agglomeration can have a considerable increasing effect on the particle size, which originally was disregarded. The effect of crystal agglomeration is accounted for in two manners. First, assuming the particles agglomerate ideally, this will yield agglomerates with a density identical to that of the constituting particles: $\rho = 2.2\ g \cdot cm^{-3}$. They will have a diameter of $(n)^{1/3} \cdot D_s$, with D_s the diameter of the single particles. Second, with the more realistic situation where the particles do not agglomerate ideally and the agglomerates have inclusions of solute and organic material [17], resulting in a lower density, it is here assumed to be $1.5\ g \cdot cm^{-3}$. The diameter of the agglomerates formed is then $(n)^{1/3} \cdot (\rho_{com}/\rho_{agg.})^{1/3} \cdot D_s$.

Experimental urolithiasis. Crystal deposits in the rat kidney were induced as previously described by either the administration sodium oxalate [18] or ethylene glycol [19]. The kidneys were fixed by perfusion and studied by light microscopy and scanning electron microscopy.

Calculations

Single crystal scenario. In the worst case scenario, when all the oxalate is precipitated into one single particle, the maximum single particle size reached (D_s) can be calculated using formula (1). T is the transit time for the particle from the site where nucleation starts to the papillary tip. Under normal oxalate loading conditions, nucleation starts in the ascending loop of Henle and T varies from 93 to 180 seconds, depending on the length of the nephron and daily urinary volume. With acute high oxalate loading nucleation may already start in the proximal tubule and T varies from 117 to 244 seconds. If A is the amount of oxalate excreted in the urine in mmoles per day, B is the number of ducts of Bellini per papilla and C is the number of papilla per kidney, then the amount of oxalate present per collecting duct is $A \cdot T/B \cdot C \cdot 90 \cdot 1440$ (90 being the molecular weight of oxalate and 1440 being the number of minutes per day).

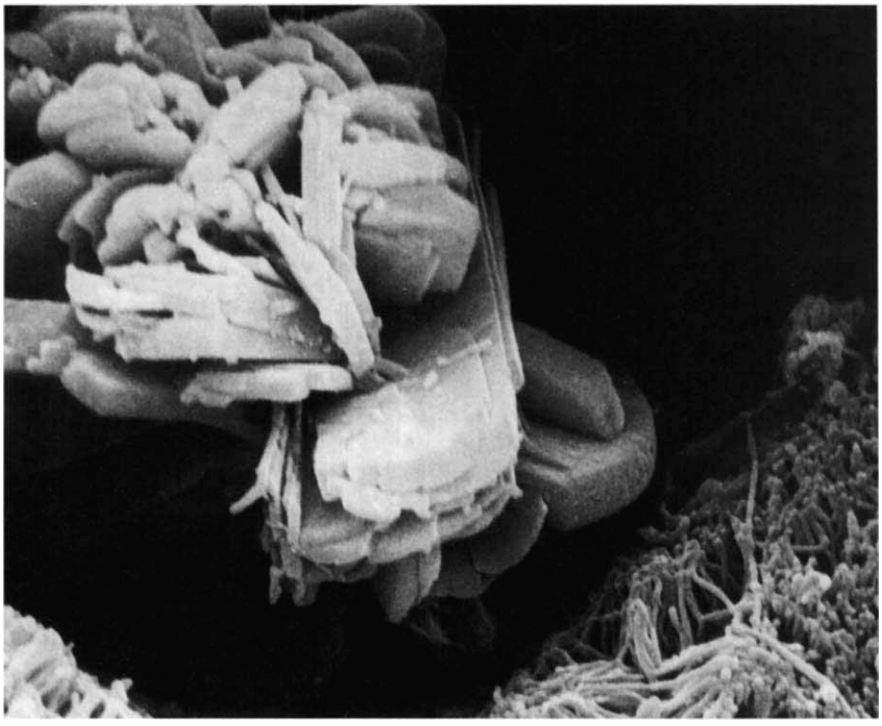

Fig. 1. *An aggregate of calcium oxalate monohydrate crystals present in the proximal tubule of the kidney of a male Sprague-Dawley rat, observed six hours after intraperitoneal administration of 7 mg sodium oxalate/100 g rat body weight (×7,000).*

Further, the density of whewellite, ρ, is 2.2 g/cm^3. The size of the particle formed, D_s, is:

$$D_s = 2 \cdot (1.62 \cdot A \cdot T/90 \cdot 1440 \cdot 2 \cdot (4/3) \cdot \pi \cdot \rho \cdot B \cdot C)^{1/3} \quad (1)$$

If A = 0.44 mmoles, T = 3.4 min., B = 40 and C = 8, then D_s = 59.4 μm. The ratio of molecular weights of whewellite and oxalate is 1.62. The numerator is divided by 2 because A is the output of two kidneys. However, as was discussed above, not all oxalate will precipitate out and the amount of oxalate present may vary under different conditions. When you correct for these factors, the maximum attainable size will be 54.6 μm, 72 μm and 103 μm under respectively the low normal, high normal and acute high scenario. In this worst case scenario, with all material going into one single crystal, the sizes reached would thus be large enough for the particles to be retained in the collecting ducts. In reality, however, the material will not be concentrated into one single crystal, but rather be divided over several crystals.

Multi-crystal scenario, no agglomeration. When the total amount of oxalate is distributed over n crystals, the size of the individual particles will maximally be $D_s \cdot (n)^{-1/3}$. The number n changes depending on the crystalluria scenario and the transit time (Table 1). If all particles are growing at the same rate, the maximum single particle size under the three crystalluria scenarios would be respectively 16.9, 10.2 to 13.1 and 8.1 to 10.4 μm (low normal oxalate), 22.3, 13.5 to 17.3 and 10.7 to 13.7 μm (high normal), 31.9, 19.2 to 24.7 and 15.3 to 19.6 μm (high acute). Still, these single crystal sizes are not realistic because of the restriction on the particle size posed by the velocity of the crystal growth process itself. In the normal scenario, where nucleation is assumed to occur at the end of the descending loop of Henle, the particle has maximally 180 seconds to grow. A particle growing at the maximum uninhibited rate of 2×10^{-4}cm per min can thus reach 6 μm. At 50% inhibited rate this will be 3 μm. Under acute oxalate

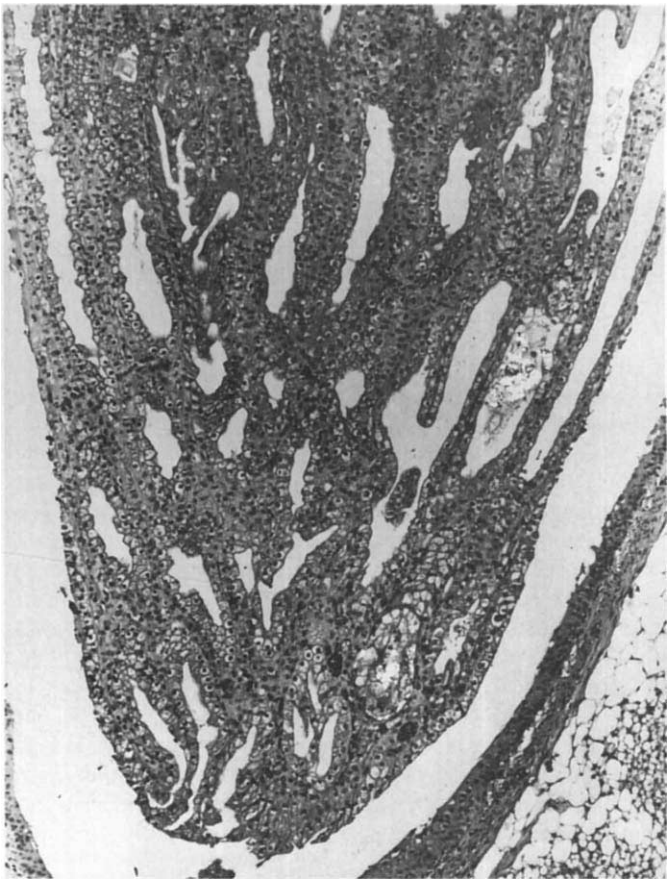

Fig. 2. *Histological section of a rat kidney after two weeks of consumption of 0.75% ethylene glycol and 2% ammonium chloride.* Calcium oxalate crystals are restricted to only a few renal tubules (×110).

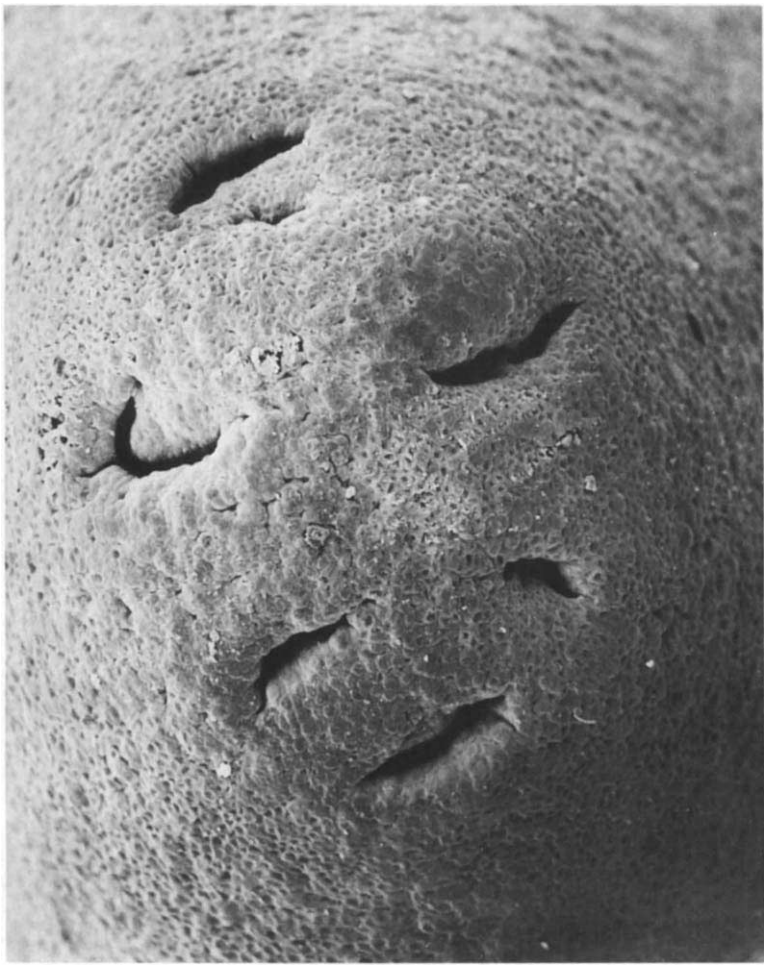

Fig. 3. *Renal papillary tip of a normal male Sprague-Dawley rat, illustrating slit-like openings of the ducts of Bellini which are 7 to 23 μm wide and 60 to 120 μm long (×210).*

loading one might expect nucleation to start in the proximal tubule and the maximum attainable size for a single particle will be 4.1 μm at the papillary tip with 50% growth inhibition. Thus, when the particles remain single, the maximum size reached will be much smaller than the inner diameter of the duct of Bellini.

Multi-crystal scenario, agglomeration occurring. The picture becomes different when the particles formed are allowed to agglomerate. If we assume the crystals to grow in a cubic form of 3 μm (normal oxalate load, long loops, volume 0.6 liter) and then agglomerate ideally, the particles formed would be 18.4 or 23.2 μm (50% scenario) large (Table 2). The packing in the agglomerate, however, will not be ideal. Synthetic agglomerates show much lower densities than 2.2 due to the inclusion of solute [20]. Crystalline material in the tubules is invariably associated with organic material and/or cell debris [21]. If we assume an agglomerate density of 1.5 (compared to 2.2 for pure whewellite and ± 1 for synthetic agglomerates) the particles formed reach sizes of 20.9 and 26.4 μm, respectively. Under acute oxalate loading, where the single particles form in the proximal tubule and have more time to grow, the maximum size of the agglomerates will be 25 and 31.5 μm, respectively. The specific values fot the different scenarios are given in Table 2.

Discussion

When Finlayson and Reid published their calculation data some 20 years ago, it provided very convincing evidence that kidney stones cannot start through a free particle mechanism, but that somewhere particle retention due to adhesion or disturbed urine flow must occur. Thus the crystallization of COM in urine from stone formers would not be expected to behave different from that in urine of healthy subjects. With respect to supersaturation, nucleation and crystal growth, the available data support this conclusion. Though COM supersaturation of stone former urine may on average be higher [21–23] and crystalluria occurs more often [2–4, 24, 25] they are not exclusive for stone formers. Also, crystal growth inhibition in stone former urine, on average, may be significantly lower [10], but the range overlaps completely with that found for non-stone forming subjects. Overall the differences in the occurrence of urinary supersaturation, crystalluria or the rate of crystal growth are not sufficient to explain the occurrence of stone formation [23]. Urine from stone formers can only be clearly distinguished from those of healthy subjects in their effects on crystal agglomeration. Though crystalluria is common in both stone formers and healthy subjects, the stone formers tend to excrete more large particles, especially agglomerates [25]. When precipitation is induced in urine, stone former urine produces more agglomerates [26]. The inhibition of crystal agglomeration is significantly reduced in stone former urine [10, 27], with a large percentage of the stone former population showing values below the lower limit of normal [10]. Furthermore, the reduction in agglomeration inhibition is directly related to the severity of the disease [10]. Finally, some of the high molecular

Table 2. Maximum particle sizes during passage through nephron

Segment	P	DLH	ALH	D	OMCD	IMCD	DOB
Normal oxalate load							
Inner D. μ	25–35	14–37	19–29	28–30	30–35	35–60	60–100
Precip.	no	no	no	yes	yes	yes	yes
G.t.[a]							
s *sec*	0	0	33	64	92/105/106	93/108/110	93/109/113
m *sec*	0	0	67	98	126/139/140	127/142/144	127/143/147
l *sec*	0	0	100	131	159/172/173	160/175/177	160/176/180
D, single							
s μ	0	0	0.6	1.1	1.5/1.8/1.8	1.5/1.8/1.8	1.6/1.8/1.9
m μ	0	0	1.1	1.6	2.1/2.3/2.3	2.1/2.4/2.4	2.1/2.4/2.5
l μ	0	0	1.7	2.2	2.7/2.9/2.9	2.7/2.9/2.9	2.7/2.9/3.0
D,aggl.[b]							
s μ	0	0	0.6	1.1	1.5/1.8/1.8	2.6/3.3/3.3	6.5/7.9/8.3
m μ			1.1	1.6	2.1/2.3/2.3	4/5.8/5.9	10/11.6/12.1
l μ			1.7	2.2	2.7/2.9/2.9	5.5/6.2/6.3	13.9/15.6/16.1
D, 50%[c]							
s μ	0	0	0	0	2.9	8	20.3
Acute oxalate load							
Inner D. μ	25–35	14–37	19–29	28–30	30–35	35–60	60–100
Precip.	yes	yes	yes	yes	yes	yes	yes
G. time[a]							
s *sec*	24	24	57	88	116/129/130	117/132/134	117/133/137
m	24	44	111	142	170/180/184	171/186/188	171/187/191
l	24	64	164	195	223/236/237	224/239/241	224/240/244
D,single							
s μ	0.4	0.4	1	1.5	1.9/2.2/2.2	2/2.2/2.2	2/2.2/2.3
m μ	0.4	0.7	1.9	2.4	2.8/3/3.1	2.9/3.1/3.1	2.9/3.1/3.2
l μ	0.4	1.1	2.7	3.3	3.8/3.9/4	3.7/4/4	3.8/4/4.1
D,agg.[b]							
s	0.4	0.4	1	1.5	1.9/2.2/2.2	3.4/4.0/4.0	8.4/9.6/10.2
m	0.4	0.7	1.9	2.4	2.8/3/3.1	5.5/6.2/6.2	13.8/15.2/15.8
l μ	0.4	1.1	2.7	3.3	3.8/3.9/4	7.7/8.6/8.6	19.8/21.3/22
D, 50%[c]							
l μ	0	0	0	0	4	10.9	27.7

Abbreviations are in Table 1.
[a] Three different situations are given, based on daily urine volumes of 16.7, 1.44 or 0.6 liters, respectively.
[b] When the crystals are evenly distributed over the nephrons, the first agglomerates are expected in the inner medullary collecting ducts.
[c] Assuming that only 50% of the ducts contain crystals, only calculated for the long loops, low volume scenario.

weight compounds from stone formers have a diminished inhibitory effect on crystal agglomeration [28, 29]. If stone formation is caused by a fixed particle mechanism solely, how can these data be explained? This apparent discrepancy prompted us to review the original calculations and now incorporate the size increasing effects of crystal agglomeration. From our results it is clear that this size-increasing effect has serious implications. When we assume even distribution of the particles formed over the collecting ducts, the particle sizes which can be reached through nucleation, growth and agglomeration combined, fall within the size range of the tubule itself. This makes retention of particles through sheer size a feasible mechanism. The maximum attainable particle size becomes even bigger when we consider that during crystalluria not all ducts contain crystals (Table 2). Finlayson and Reid pointed out that these calculations are sensitive to small changes in the transit time. This becomes obvious when we compare the sizes obtained in nephrons with short loops of Henle to those obtained in nephrons with long loops of Henle. Further, it seems likely that solid particles will not flow with the same rate as the urine-fluid but at a slower rate. Each time a particle hits the tubular walls, its velocity will be reduced. The extent of this will depend on how strong the interaction is between the particle and the cell-membrane. In this respect it is interesting to note that, using red blood cells, COM crystals were found to interact much stronger with the membrane than calcium oxalate dihydrate crystals [30]. It remains to be established whether this effect is also seen with kidney cells and whether in this respect single crystals and agglomerates behave differently. The chance that particles will be retained by adhering to epithelium will be especially higher whenever a bend in the tubule is encountered or when two tubular flows combine. The highest risk for forming large agglomerates will be at the end of the collecting ducts and in the duct of Bellini where single particle sizes are biggest and crystal concentration is highest. From plastic casts made of post-mortem human kidneys, it appears that distal tubules and collecting ducts show acute angles (70°) and z-bends [31]. These aberrations are more prominent at the bases of the papillae in the lower calices. An intriguing aspect was that these acute angles and bends were located at the same site where so-called "laminated seed-stones" were concentrated. These were stones with the usual lamination of crystalline and organic material and one or two crystalline centers.

Overall, under the realistic assumptions made here, a normal oxalate excretion of 0.44 mmoles per day, 50% inhibited crystal growth and a relative supersaturation of 5, the particles formed, especially agglomerates, have a fair chance of being retained through their size. This can actually be seen when calcium oxalate

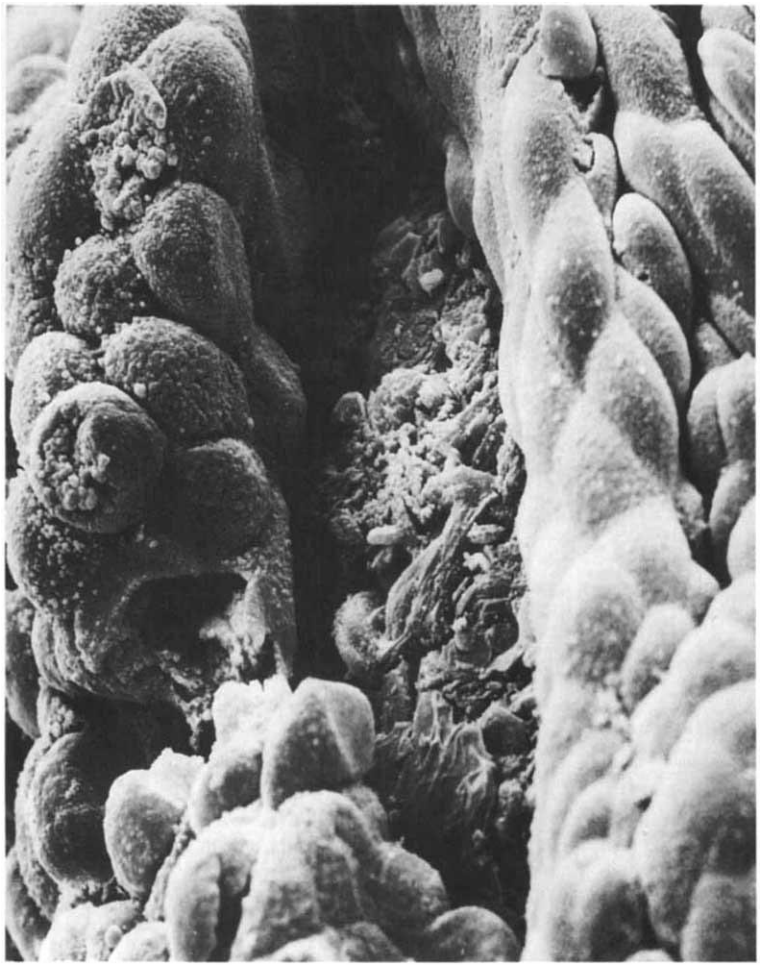

Fig. 4. *Opening of a duct of Bellini at the renal papillary tip of a male Sprague-Dawley rat.* Opening is occluded by aggregates of calcium oxalate monohydrate crystals observed 6 hours after the intraperitoneal administration of 7 mg sodium oxalate/100 g rat body weight (×1,800).

nephrolithiasis is experimentally induced in rats. Large agglomerates are found blocking the nephron at the papillary tip (Fig. 4). When acute hyperoxaluria is induced in rats by intraperitoneal injection of sodium oxalate or dietary administration of 1% ethylene glycol, nephrolithiasis ensues [9, 13, 32] and agglomerates are even found in the proximal tubule (Fig. 1) [9]. The structure of the stones found in the renal pelvis under these conditions suggested that tubular deposits agglomerated to form the nidus of the stone [32]. The danger of acute hyperoxaluria, for example after a dietary load, may thus be that the nucleation threshold is surpassed earlier in the nephron and the particles have more time to increase in size and form the nidus of a stone. It is thus possible that particles form through nucleation, growth and agglomeration which are big enough to be retained in the collecting duct. When such a large agglomerate is retained, it will further accrue organic and inorganic material and become a stone. The whole concretion may be pushed along the nephron or if it is too big to move further, increase in size until it breaks into the pelvic area. When the particle is pushed along, it may damage the cells lining the tubule and reach the basement membrane to become attached there [18]. This could explain the reported frequent occurrence of tubular remnants inside urinary stones [33].

In conclusion, the calculations presented here support the theory that formation and subsequent retention of large particles because of aberrance in calcium oxalate crystallization processes, especially crystal agglomeration, may be the crucial first steps leading to stone formation. The model described can be used to evaluate the effects of changes in nephron morphology.

Acknowledgments

Dr. Kok is the recipient of a Royal Dutch Academy of Sciences Research Fellowship. This work was partially supported by NIH grants #5RO1 DK41434-02 and 5PO1 DK20586-16.

Reprint requests to Dirk J. Kok, Department of Endocrinology, Academic Hospital Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

References

1. Finlayson B, Reid F: The expectation of free and fixed particles in urinary stone disease. *Invest Urol* 15:442–448, 1978
2. Robertson WG: A method for measuring calcium crystalluria. *Clin Chim Acta* 26:105–110, 1969
3. Elliot JS, Rabinowitz IN: Calcium oxalate crystalluria. *J Urol* 116:773–775, 1976
4. Leskovar P, Hartung R, Santoso I: Kristalluria, mikroerythruria und proteinuria: Haufigkeit des vorkommens und des koinzidenz. *Fortschr Urol Nephrol* 11:307–315, 1978

5. KAISSLING B, KRIZ W: Structural analysis of the rabbit kidney, in *Advances in Anatomy, Embryology and Cell Biology*, 1979, p. 56
6. TISELIUS HG, ALMGARD LE: The diurnal excretion of oxalate and the effect of pyridoxine and ascorbate on oxalate excretion. *Eur Urol* 3:41–46, 1977
7. AHLSTRAND C, LARSSON L, TISELIUS HG: Variations in urine composition during the day in patients with calcium oxalate stone disease. *J Urol* 131:77–81, 1984
8. BALCKE P, ZAZGORNIK J, SUNDER-PLASSMANN G, KISS A, HAUSER AC, GREMMEL F, DERFLER K, STOCKENHUBER F, SCHMIDT P: Transient hyperoxaluria after ingestion of chocolate as a risk factor for calcium oxalate calculi. *Nephron* 51:32–34, 1989
9. KHAN SR, HACKETT RL: Retention of calcium oxalate crystals in renal tubules. *Scann Micr* 5:707–712, 1991
10. KOK DJ, PAPAPOULOS SE, BIJVOET OLM: Crystal agglomeration is a major element in calcium oxalate urinary stone formation. *Kidney Int* 37:51–56, 1990
11. PITTS RF: *Physiology of the Kidney and Body Fluids.* Chicago, Year Book Medical Publishers Inc, 1964
12. MALVIN RL, WILDE WS, SULLIVAN LP: Localization of nephron transport by stop flow analysis. *Am J Physiol* 194:135–142, 1958
13. STEINHAUSEN M, WAYLAND H, FOX JR: Quantitative analysis of tubular passage of FITC-dextrans in kidneys of rats. *Pflügers Arch* 369:273–279, 1977
14. COE FL, PARKS JH: Defenses of an unstable compromise: Crystallization inhibitors and the kidney's role in mineral regulation. *Kidney Int* 38:625–631, 1990
15. KHAN SR, HACKET RL: Calcium oxalate urolithiasis in the rat: Is it a model for human stone disease? A review of recent literature. *Scann Electr Micr* II:759–774, 1985
16. OLIVER J, MCDOWELL M, WHANG R, WELT LG: The renal lesions of electrolyte imbalance IV. The intranephronic calculosis of experimental magnesium depletion. *J Exp Med* 124:263–278, 1966
17. KHAN SR, HACKETT RL: Role of organic matrix in urinary stone formation: An ultrastructural study of crystal matrix interface of calcium oxalate monohydrate stones. *J Urol* 150:239–245, 1993
18. KHAN SR, FINLAYSON B, HACKETT RL. Experimental calcium oxalate nephrolithiasis, role of renal papilla. *Am J Pathol* 107:59–69, 1982
19. KHAN SR, SHEVOCK PN, HACKETT RL: Urinary enzymes and calcium oxalate urolithiasis. *J Urol* 142:846–849, 1989
20. BLOMEN LJMJ: Growth and agglomeration of calcium oxalate monohydrate. Thesis, Leiden University, 1982
21. PAK CYC, HOLT K: Nucleation and growth of brushite and calcium oxalate in urine of stone formers. *Metabolism* 25:665–673, 1993
22. MARANGELLA M: Urine saturation with calcium salts in normal subjects and idiopathic stone formers. Estimated by an improved computer model system. *Urol Res* 13:189–193, 1985
23. KOK DJ, PAPAPOULOS SE: Physicochemical considerations in the development and prevention of calcium oxalate urolithiasis. *Bone Mine* 25:665–673, 1993
24. WERNESS PG, BERGERTS JH, SMITH LH: Crystalluria. *J Cryst Growth* 53:166–181, 1981
25. ROBERSTON WG, PEACOCK M, NORDIN BEC. Calcium crystalluria in recurrent renal stone formers. *Lancet* ii:21–24, 1969
26. PRIEN EL JR: Calcium oxalate renal stones. *Annu Rev Med* 26:173–179, 1975
27. KOK DJ, PAPAPOULOS SE, BIJVOET OLM: Excessive crystal agglomeration with low citrate excretion in recurrent stone formers. *Lancet* i:1056–1058, 1986
28. HESS B, NAKAGAWA Y, COE FL: Inhibition of calcium oxalate monohydrate crystal aggregation by nephrocalcin, a urinaryglycoprotein crystal growth inhibitor: Comparison to Tamm Horsfall glycoprotein, albumin and beta2-microglobulin. *Am J Physiol* 257:216–232, 1982
29. HESS B: Tamm Horsfall glycoprotein—Inhibitor or promoter of calcium oxalate monohydrate crystallization processes? *Urol Res* 20:83–86, 1992, 1993
30. WIESSNER JH, MANDEL GS, MANDEL NS: Membrane interactions with calcium oxalate crystals: Variation in hemolytic potentials with crystal morphology. *J Urol* 135:835–839, 1986
31. GRAVES FT: An experimental study of the anatomy of the tubules of the human kidney and its relation to calculus formation. *Brit J Urol* 54:569–574, 1982
32. RUSHTON HG, SPECTOR M, RODGERS AL, HUGHSON M, MAGURA CE: Developmental aspects of calcium oxalate tubular deposits and calculi inducedin rat kidneys. *Invest Urol* 19:52–57, 1981
33. CIFUENTES D, MEDINA JA, MIÑÓN CIFUENTES JLR, CIFUENTES I: Proportion of papillary stones among 400 spontaneous stones. *Fortschr Urol Nephrol* 23:290–292, 1985